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Title: Variation in herbivory-induced responses within successively flushing *Quercus serrata* seedlings under different nutrient conditions

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Abstract Herbivore damage can induce the host plant to alter the chemical and physical quality of its leaves, which is thought to be a plant strategy for avoiding further herbivory, termed induced response. In woody plants, many studies have considered variation in induced responses with resource availability, but few studies have examined this variation in relation to growth patterns of woody plants. We studied phenotypic variability of induced response within successively flushing *Quercus serrata* seedlings. *Q. serrata* seedlings were grown under controlled conditions. The controlled factors were herbivore damage (herbivore-damaged and -undamaged) and soil fertility (low and high). At each flush stage, concentrations of condensed tannin (CT), total phenolics (TP), and nitrogen (N) in leaves were analysed and leaf mass per area (LMA) was measured. CT and TP concentration of leaves and LMA were higher in herbivore-damaged seedlings. Leaves of the first flushes showed greater sensitivity to herbivore damage and had a higher CT concentration than leaves of the later flushes. Furthermore, seedlings growing in low-fertility soil showed greater induced response. The results suggest that *Q. serrata* seedlings showed induced response related to contributions of the tissue to current productivity. Leaves of the first flush showed greater induced response possibly because they play an important role in subsequent growth. The potential of Q. serrata seedlings to adjust the properties of leaves depending on herbivory and soil fertility in relation to growth patterns may be advantageous in forest floor where seedlings grow in heterogeneous soil fertility and are constantly exposed to herbivory.

Keywords: Herbivorous insect, Induced response, Oak, Phenolics, Leaf flushing, Soil fertility

Introduction

Leaf quality of host plants is an important property for herbivores in choosing their food (e.g., Mattson 1980; Nykänen and Koricheva 2004). Leaves with high nitrogen (N) concentration have been positively related to insect feeding and performance (Wait et al. 1998; Lower et al. 2003). Concentrations of nitrogen and phenolic compounds within leaves are major determinants of distribution and abundance of herbivores (e.g., Kytö et al. 1996; Lill and Marquis 2001; Murakami et al. 2005). For example, condensed tannin (CT) and total phenolics (TP) have negative effects on the growth and survival of insects (e.g., Mutikainen et al. 2000; Lill and Marquis 2001; Nomura and Itioka 2002). Leaf mass per area (LMA) and leaf toughness are often used as indices of leaf physical defences (e.g., Feeny 1970; Kudo 1996; Nabeshima et al. 2001) and may vary in relation to leaf chemical properties (Poorter et al. 2009). Furthermore, LMA and leaf toughness are recognised as important deterrents to herbivory (Coley 1983; Reich et al. 1991; Choong 1996, Hanley et al. 2007).

Conversely, herbivores can affect the quality of damaged leaves and leaves produced after implementation of the damage (e.g., Karban and Myers 1989; Karban and Baldwin 1997).

Consequently, in a seasonal environment, leaf damage early in the growth season affects the distribution and abundance of herbivores, which, in turn, causes changes in leaf quality later in the growth season (Hunter 1987; Wold and Marquis 1997; Boege 2004). These changes in leaf quality caused by herbivory thus seem to be one of important plant strategies for avoiding additional herbivore damage; this strategy is termed "induced response" (e.g., Karban and Myers 1989; Karban and Baldwin 1997).

Induced response varies with environmental factors such as the availability of nutrients, light and water (e.g., Hunter and Schultz 1995; Mutikainen et al. 2000; Nabeshima et al. 2001; Baraza et al. 2004). The pattern of carbon allocation toward induced response depends not only on the external availability of resources, but also on the internal characteristics of the plant (Nykänen and Koricheva 2004). Nykänen and Koricheva (2004) conducted a meta-analysis of 68 studies, and showed that the plant responses induced by herbivory depend on plant type (evergreen or deciduous) and inherent growth rate; phenolic compounds of leaves increase following damage in deciduous and fast-growing species, but not in evergreen or slow-growing species. Therefore, in clarifying induced response in woody species, it is important to take into consideration the variations in leaf quality within plant.

74 Several studies on *Quercus* species have shown a close connection between leaf quality
75 and the abundance and performance of herbivorous insects (e.g., Feeny 1970; Rossiter et al.
76 1988; Hunter and Schultz 1995; Forkner et al. 2004). In Japan, *Quercus* species, including *Q.*
77 *serrata,* are important components of deciduous temperate forests (Ozawa et al. 2000; Ohsawa
78 et al. 2008) and have been the subject of understanding interactions between plants and
79 herbivores. Large numbers of herbivorous insects, such as Lepidopterans use *Q. serrata* as a
80 host plant (Teramoto 1993; Teramoto 1996). The extent of leaf damage by herbivorous insects
81 in *Quercus* species changes temporally among years (Furuno and Saito 1981) and spatially
82 among branches, even within the same individual (Yamasaki and Kikuzawa 2003; Nakamura et
83 al. 2008). Like other woody plants, *Quercus* species could respond to the temporal and spatial
84 fluctuations in herbivore damage because they have semi-autonomously modular units (Watson
85 1986; Sprugel et al. 1991). *Quercus* seedlings growing in forest floor may respond especially
86 well to severe herbivore damage by adjusting the pattern of leaf flushing because they are
87 constantly exposed to insect attacks during most of growing season.

88 In this study, we focused on induced response in relation to growth characteristic of
89 seedlings of *Q. serrata*. *Quercus* seedlings/saplings can show successively flushing, which can

show several growth flushes within a growing season (e.g., Borchert 1975; Charr et al. 1997a, b). We previously demonstrated that herbivore damage increased both the total number of flushes and the probability of producing a later flush in *Q. serrata* seedlings (Mizumachi et al. 2004, 2006). Our previous studies also showed that the probability of flushing was prominent in seedlings grown in high-fertility soil than ones grown in low-fertility soil (Mizumachi et al. 2004, 2006). While these studies demonstrated the nutrient level dependent effect of herbivory on the pattern of flushing, induced response to herbivory at different nutrient levels in *Q. serrata* remains to be known. Here, we investigated phenotypic variability of induced response within *Q. serrata* seedlings. We examined whether herbivory induces change in chemical and physical properties of leaves in different flushes, and whether the response varies depending on the soil fertility. The results will be discussed in relation to the growth pattern under different soil nutrient availability.

Materials and Methods

105 Plant materials

106

107 We used a group of 120 *Quercus serrata* Thunb. Ex Murray seedlings purchased from
108 the Kutsuki Village Forest Association (Shiga Prefecture, Japan) in December 2001. These
109 seedlings had been grown in uniform environment prior to the experiment. The roots of each
110 seedling were washed to remove any remnants of soil, and then all seedlings were transplanted
111 into plastic pots (44 cm in diameter, 24 cm in depth) with 500 ml of kanuma soil (pumice) at the
112 bottom and filled with sand. The mean seedling height at the start of the experiment was 43.4 ±
113 0.4 cm (mean ± SE). Seedling height did not differ significantly among the treatments (Scheffe's
114 range test, $P > 0.05$). All seedlings were watered to saturation for 10 min daily by an automatic
115 sprinkler (Sprinkler Thinker DC-1, Irrigation Control Equipment, Galcon®).

116 In early April 2002, the winter buds of *Q. serrata* seedlings were beginning to unfold
117 (defined as 'first flush'). These shoots that elongated from the winter buds were termed 'the first
118 shoots'. During the growth season, most of the seedlings had more than one flush. The shoots
119 formed after the first flush were considered 'the second shoots', and the following shoots were
120 'the third shoots' and 'the fourth shoots'. These flushes and shoots emerging after the first flush

were defined as ‘later flush’ and ‘later shoots’, respectively. Bud-break of the last flush occurred in early October 2002. Because a two-dimensional diagram was drawn to illustrate the branching structure of each seedling when new shoots elongated during the growing season, we could identify when each shoot elongated. Maximum number of flushes was five among all seedlings and four among seedlings sampled.

Experimental design

The study was carried out at Kitashirakawa Experimental Station of Kyoto University in Kyoto, Japan (35.02°N, 135.47°E). The average annual temperature at the station is 15.9°C (Field Science Education and Research Center, Kyoto University). *Quercus serrata* is found naturally distributed around the experimental station. Seedlings were grown in two greenhouses (H1, 10 × 7.5 m, 4 m in height; H2, 9.5 × 4.4 m, 3.5 m in height). These greenhouses were located on the same site with no obvious difference in environmental conditions and the distance between two greenhouses was within 25m. Therefore, we have treated the data of the greenhouses equally for analyses. The roofs were made of transparent plastic. The sides were

137 constructed using nylon mesh to allow free air circulation. In this experiment, we manipulated
138 herbivore damage and soil fertility, but we did not manipulate temperature and light conditions
139 in the greenhouses.

140 We controlled the herbivore damage by size of nylon mesh. The sides of the
141 herbivory-undamaged blocks were made of 1 × 1 mm nylon mesh. This mesh size effectively
142 reduced herbivore damage (the leaf area loss in herbivore-undamaged blocks were less than 3%).
143 Because a few insect invasions did occur, we checked all seedlings and removed invasive
144 insects from the herbivory-undamaged blocks every 2 days. On the other hand, those in the
145 herbivory-damaged blocks were made of 20 × 20 -mm mesh to allow insect herbivores free
146 access. *Quercus serrata* and other *Quercus* species (e.g. *Q. glauca* and *Q. acutissima*)
147 are growing also outside the greenhouses. Therefore we can regard that the seedlings in
148 these blocks were damaged naturally by insect herbivores.

149 Leaf damage within this experimental system was caused mainly by the following
150 generalist herbivorous insects: larvae of Lepidoptera belonging to the families Oecophoridae,
151 Lecithoceridae, Noctuidae, Geometridae, Lymantriidae, and Arctiidae; larvae of Hymenoptera
152 belonging to the family Tenthredinidae; and adults of Coleoptera belonging to the families

153 Attelabidae and Scarabaeidae (Ishii H and Osawa N, unpublished data).

154 Seedlings in each block were randomly assigned to fertilisation treatments (low- and
155 high-soil fertility) with the application of 25:5:20 NPK fertiliser (Peters Professional,
156 HYPONeX JAPAN®) every 2 weeks from April to November 2002. The concentration of
157 fertiliser was adjusted to obtain two levels of soil fertility. Half of the seedlings in each block
158 were grown under low soil fertility (20 kg N ha^{-1} $year^{-1}$); the other half grew under high soil
159 fertility (200 kg N ha^{-1} $year^{-1}$).

160

161

162 Plant measurements

163

164 A two-dimensional diagram was drawn to illustrate the branching structure of each
165 sapling when new shoots elongated during the growing season. For each shoot, all leaves were
166 roughly categorized into one of seven classes based on leaf damage, which was determined
167 visually by estimating the percentage of leaf area loss: 0%, damage class 0; 1-5 %, damage class
168 1; 6-25 %, damage class 2; 26-50 %, damage class 3; 51-75 %, damage class 4; 76-99 %,
169 damage class 5; 100 %, damage class 6. The assessment of leaf damage class was done when

each shoot has stopped elongating and the leaves have just fully unfolded. Leaf damage for each shoot was calculated as an average value of these damage classes.

In early October 2002, we randomly selected three seedlings per treatment (12 seedlings in total) for analysing chemical and physical properties of leaves. The experimental system was planned for continuous research; therefore, we sampled a minimum number of seedlings. The leaves of each flush within sampled seedlings were photocopied separately. The images were scanned and then processed with an image analysis program (NIH image ver. 1.63, National Institutes of Health, MD, USA) to obtain leaf area of each shoot. After photocopying, the leaves were oven-dried at 70°C for 2d. Dry mass of leaves was measured to determine the leaf mass per area (LMA). Dried leaves were ground into fine powder with a mill (TI-100, CMT CO. LTD., Tokyo, Japan). The nitrogen (N) concentration was determined by gas chromatography with an NC analyser (SUMIGRAPH, NC-900, SUMIKA Chemical Analysis Service, LTD., Tokyo, Japan). The shoots analysed were: (1) the first shoots (120 for LMA, and 100 for N), (2) the second shoots (50 for LMA, 36 for N), and (3) the third and fourth shoots (43 for LMA, 33 for N). The nitrogen analysis required at least 20 mg of dried leaf, and therefore we could not analyse several small shoots.

186 For each seedling, we used five first shoots and the subsequent shoots produced from the
187 five first shoots for analyses of condensed tannin (hereafter CT) and total phenolics (hereafter
188 TP). Because several shoots yielded small amount of leaves for chemical analyses, we could not
189 analyse all selected shoots. The categories of shoots analysed included (1) the first shoots (53 for,
190 CT and 56 for TP), (2) the second shoots (27 for CT, 27 for TP), and (3) the third and fourth
191 shoots (22 for CT, 23 for TP). The sample leaf powders were extracted with 50% methanol for
192 24 h. The concentration of CT was quantified with a spectrophotometer (UV-1200,
193 SHIMADZU, Kyoto, Japan), using cyaniding chloride as a standard (Porter et al. 1986). The
194 concentration of TP was quantified with a spectrophotometer (U-1000, HITACHI,
195 Tokyo, Japan), using tannic acid as a standard (Waterman and Mole 1994). Phenolic
196 compounds, such as CT and TP, in leaves have been commonly used as indicators of chemical
197 defence against herbivores (e.g., Feeny 1970; Forkner and Hunter 2000; Mutikainen et al. 2000;
198 Nabeshima et al. 2001; Forkner and Marquis 2004; Matsuki et al. 2004; Murakami et al. 2005;
199 Koike et al. 2006).
200 As a preliminary analysis of initial properties of selected seedlings, we compared the
201 lengths of the first shoots among sampled seedlings within each treatment. A one-way analysis

of variance (ANOVA) revealed no noticeable differences among individuals within the same treatment (one-way ANOVA, herbivory-damaged under low soil fertility, $F_{2,12} = 0.500$, $P = 0.619$;, herbivory-undamaged in low-fertility soil, $F_{2,12} = 1.619$, $P = 0.239$; herbivory-undamaged under high soil fertility, $F_{2,10} = 2.071$, $P = 0.177$), except for one treatment (herbivory-damaged in high-fertility soil, $F_{2,12} = 9.724$, $P = 0.003$). In this treatment, several first shoots were eaten by insect herbivores before elongation finished, and the difference among individuals is thus likely to reflect the damage by herbivory rather than intrinsic variation among the seedlings. Accordingly, we analysed the data without distinction among individuals within each treatment.

Statistical analysis

For each flush stage, differences in leaf qualities were tested using a two-way ANOVA with two between-subject factors (herbivore damage and soil fertility). However, the third and the fourth shoots were tested together, because the harvested seedlings had few flush-stage shoots. When a significant interaction was detected between herbivore damage and soil nutrient fertility, Tukey's HSD test was performed for comparisons among the four treatments.

For each treatment, differences in CT concentration and LMA between the periods of shoot production (the first shoots and the later shoots) were tested using Student's *t*-test. CT concentration and LMA scarcely decreased as the season progressed, while N concentration and TP concentration were unstable over the growing season (Salminen et al. 2004; Migita et al. 2007). For this reason, we did not make comparisons of N concentration and TP concentration in leaves between periods of shoot production.

The leaf damage of each flush-stage was analysed with Wilcoxon rank sum test under low and high soil fertility. All statistical analyses were performed with JMP ver. 6.0 software (SAS Institute 2005).

Results

Condensed tannin concentration

For leaves of the first shoots, the effects of herbivore damage and soil fertility on the condensed tannin (CT) concentration were significant (two-way ANOVA, herbivore damage, *F*

235 $_{1,49}$ = 35.712, P < 0.0001; soil fertility $F_{1,49}$ = 15.276, P = 0.0003). The CT concentration was
236 significantly higher in herbivore-damaged seedlings than in undamaged seedlings, and
237 was significantly higher in seedlings in low-fertility soil than in high-fertility soil (Fig.
238 1). There was no significant interaction between herbivore damage and soil fertility
239 (two-way ANOVA, $F_{1,49}$ = 0.314, P = 0.578, Fig. 1). For leaves of the second shoots,
240 the effects of herbivore damage on CT concentration were significant (two-way
241 ANOVA, $F_{1,23}$ = 10.272, P = 0.004). The CT concentration was significantly higher in
242 herbivore-damaged seedlings than in undamaged seedlings (Fig. 1). The effects of soil
243 fertility on CT concentration were not significant (two-way ANOVA, $F_{1,23}$ = 0.024, P =
244 0.878, Fig. 1). There was no significant interaction between herbivore damage and soil
245 fertility (two-way ANOVA, $F_{1,23}$ = 0.155, P = 0.697, Fig. 1). For leaves of the third
246 and fourth shoots, CT concentration did not differ among the four treatments (two-way
247 ANOVA, $F_{1,18}$ = 2.862, P = 0.066, Fig. 1).

248 In herbivore-undamaged seedlings in high-fertility soil, the CT concentration of leaves
249 was not significantly different between flush periods (Table 1). In the other treatments, CT
250 concentration of leaves in the first shoots was higher than in the later shoots (Table 1).

251

252 Total phenolics concentration

253

254 For leaves of the first shoots, the effects of herbivore damage and soil fertility on the total
255 phenolics (TP) concentration were significant (two-way ANOVA, herbivore damage, $F_{1, 52}$ =
256 4.800, P = 0.033; soil fertility, $F_{1, 52}$ = 24.909, P < 0.0001). The TP concentration was
257 significantly higher in herbivore-damaged seedlings than in undamaged seedlings, and
258 was significantly higher in seedlings in low-fertility soil than in high-fertility soil (Fig.
259 2). There was no significant interaction between herbivore damage and soil fertility
260 (two-way ANOVA, $F_{1, 52}$ = 0.188, P = 0.667, Fig. 2). For leaves of the second shoots,
261 TP concentration was not significantly different among treatments (two-way ANOVA, F
262 $_{1, 23}$ = 1.850, P = 0.166, Fig. 2). For leaves of the third and fourth shoots, effects of soil
263 fertility on TP concentration were significant (two-way ANOVA, $F_{1, 19}$ = 14.415, P =
264 0.001); the TP concentration in seedlings in low-fertility soil was significantly higher
265 than in high-fertility soil (Fig. 2). The effects of herbivore damage on TP concentration
266 were not significant (two-way ANOVA, $F_{1, 19}$ = 3.129, P = 0.093, Fig. 2). There was

267 no significant interaction between herbivore damage and soil fertility (two-way
268 ANOVA, $F_{1, 19} = 0.265$, $P = 0.613$, Fig. 2).
269
270 Leaf mass per area
271
272 For leaves of the first shoots, there was significant interaction between herbivore damage
273 and soil fertility (two-way ANOVA, $F_{1, 116} = 15.751$, $P = 0.0001$, Fig. 3). The LMA in
274 herbivore-damaged seedlings was significantly higher than in undamaged seedlings,
275 especially in low-fertility soil (Fig. 3). For leaves of the second shoots, effects of
276 herbivore damage on LMA were significant (two-way ANOVA, $F_{1, 46} = 34.245$, $P <$
277 0.0001); the LMA in herbivore-damaged seedlings was significantly higher than in
278 undamaged seedlings (Fig. 3). However, the effects of soil fertility on LMA were not
279 significant (two-way ANOVA, $F_{1, 46} = 0.014$, $P = 0.908$, Fig. 3). There was no
280 significant interaction between herbivore damage and soil fertility (two-way ANOVA,
281 $F_{1, 46} = 3.691$, $P = 0.061$, Fig. 3). For leaves of the third and fourth shoots, there was
282 significant interaction between herbivore damage and soil fertility (two-way ANOVA,

283 $F_{1, 39} = 9.047$, $P = 0.005$, Fig. 3); the LMA in herbivore-damaged seedlings was
284 significantly higher than in undamaged seedlings, especially under low-fertility soil
285 conditions (Fig. 3).

286 In herbivore-damaged seedlings in low-fertility soil, LMA in the later shoots was
287 greater than in the first shoots (Table 1). In the other treatments, LMA was not
288 significantly different between flush periods (Table 1).

289

290 Nitrogen concentration

291

292 For leaves of the first shoots, the effects of soil fertility on the nitrogen (N) concentration
293 were significant (two-way ANOVA, $F_{1, 96} = 61.974$, $P < 0.0001$). The N concentration of
294 seedlings in high-fertility soil was significantly higher than in low-fertility soil (Fig. 4),
295 but the effects of herbivore damage on the N concentration were not significant
296 (two-way ANOVA, $F_{1, 96} = 1.931$, $P = 0.168$, Fig. 4). There was no significant
297 interaction between herbivore damage and soil fertility (two-way ANOVA, $F_{1, 96} =$
298 2.747, $P = 0.101$, Fig. 4). For leaves of the second shoots, the N concentration was not

299 significantly different among treatments (two-way ANOVA, $F_{1, 32} = 2.121$, $P = 0.117$,
300 Fig. 4). For the leaves of the third and fourth shoots, the effects of soil fertility on N
301 concentration were significant (two-way ANOVA, $F_{1, 29} = 18.001$, $P = 0.0002$). The N
302 concentration in high-fertility soil seedlings was significantly greater than in
303 low-fertility soil seedlings (Fig. 4). The effects of herbivore damage on the N
304 concentration were not significant (two-way ANOVA, $F_{1, 29} = 0.239$, $P = 0.629$, Fig. 4).
305 There was no significant interaction between herbivore damage and soil fertility
306 (two-way ANOVA, $F_{1, 29} = 0.524$, $P = 0.475$, Fig. 4).
307
308 Leaf damage
309
310 The leaf damage of the second shoots in high-fertility soil was significantly higher than in
311 low-fertility soil (Wilcoxon rank sum test, $P = 0.1566$, Fig. 5). While, soil fertility did not
312 affect the leaf damage of the first, third, forth or fifth shoots (Wilcoxon rank sum test, first shoot,
313 $P = 0.0099$; third shoot, $P = 0.0577$; forth shoot, $P = 0.1445$, fifth shoot, $P = 0.7582$, Fig. 5).
314

315 **Discussion**

316

317 The effect of insect damage on leaf quality at each flush stage

318

319 The increase in phenolic compounds in leaves of the herbivore-damaged seedlings and
320 the increase in LMA in every flush stage of *Quercus serrata* seedlings in this study can be
321 regarded as induced resistance against insect damage. Leaf damage by herbivorous insects
322 increased the condensed tannin (CT) concentration of leaves of the first and the second shoots,
323 and also increased the total phenolics (TP) concentrations of leaves of the first shoots (Figs. 1
324 and 2). Similar to our findings, the increases in CT and TP concentrations induced by herbivores
325 have been previously described (Haukioja 1990; Karban and Baldwin 1997; Nykänen and
326 Koricheva 2004). Increases in these phenolic compounds in leaves of a certain flush
327 stage can be interpreted as defensive reactions against further herbivory. Moreover, in
328 our study, leaf damage by herbivorous insects significantly increased the LMA in
329 every flush stage, especially when seedlings were grown in soil with low fertility (Fig.
330 3). This suggests that the increase in LMA is a response to the herbivore attacks. Our
331 result is related to findings of a previous study that showed a negative correlation

332 between LMA and defoliation by herbivores within a crown of *Fagus crenata*
333 (Yamasaki and Kikuzawa 2003).

334 We showed for *Q. serrata* seedlings that N concentration of damaged leaves did not differ
335 from that in intact leaves (Fig. 4). In contrast, Kudo (1996) reported that nitrogen (N)
336 concentration of leaves of *Q. crispula* decreased following artificial damage. The differences in
337 N concentration between damaged leaves and intact leaves were explained by differences in N
338 allocation to leaves in mid-summer; N was not allocated to damaged leaves in mid-summer,
339 although it was allocated to intact leaves in the same season (Kudo 1996). On the other hand, the
340 discrepancy between the results for *Q. serrata* and *Q. crispula* might be caused by
341 species-specific differences in regrowth processes between the two plants. *Quercus serrata*
342 seedlings produced later shoots in mid-summer (Mizumachi et al. 2004, 2006); as a result, the N,
343 which was expected to be allocated to intact leaves in mid-summer, was distributed to the later
344 shoots.

345

346 Variability of induced responses

347

348 The CT concentration varied among different flush stages, while no such
349 difference was detected for TP concentration. The CT concentration of leaves in the first
350 flush was higher than that in leaves in the later flush, except for herbivore-undamaged
351 seedlings in high-fertility soil, which showed no significant differences between flush
352 periods (Table 1). This indicates that the difference in CT concentration between flush
353 periods did not merely arise from leaf age. The difference in CT concentration between
354 flush periods could reflect contribution of different flushes to subsequent growth. For
355 example, Matsuki et al. (2004) showed that *Betula platyphylla* and *B. ermanii* invest highly in
356 defence in their early-season leaves, which make a major contribution to subsequent growth. In
357 contrast, *B. maximowicziana* invests its defences in late-season leaves, which are more
358 important to its growth (Matsuki et al. 2004). In case of successively flushing *Quercus* species,
359 leaves of the first and second shoots act as a carbon source during the production of
360 subsequent-flush shoot (Dickson et al. 2000). Therefore, leaves of the first shoots play a highly
361 important role in growth of subsequent-flush shoots (Alaoui-Sossé et al. 1996; Mizumachi et al.
362 2006). Our findings support this idea by showing that leaves of the first shoots, which would
363 contribute greatly to subsequent growth, had higher CT concentration (Table 1) and the effects

364 of herbivore damage on leaf qualities were more clearly in leaves of the later shoots (Figs. 1–3).

365 In contrast to CT concentration, TP concentration of leaves in the first shoots was not
366 higher than that of leaves in the second shoots, and the third and fourth shoots (Fig. 2). The
367 results are related to the seasonal variation in TP; concentrations of TP and hydrolyzable tannins,
368 which comprise the dominant group of phenolic compounds, are higher in younger oak leaves
369 (Rossiter et al. 1988; Mauffette and Oechel 1989; Salminen et al. 2004).

370 Our data showed that, for leaves of first flush and leaves of third and forth flush, increase
371 in CT and TP concentration in *Q. serrata* seedlings in low-fertility soil was greater than those in
372 high-fertility soil (Figs. 1-3) in accordance with several previous studies finding higher induced
373 response in low-fertility soil (Koricheva et al. 1998, Hunter and Schultz 1995; Ruohomäki et al.
374 1996; Hikosaka et al. 2005; Cornelissen and Stiling 2006; Koike et al. 2006). However leaf
375 damage observed in these flushes did not differ between low- and high-fertility soil (Fig. 5). In
376 the second flush, in contrast, leaf damage was higher in high-fertility soil than in low-fertility soil
377 (Fig. 5), while induced response did not differ between the different soil fertility levels (Figs.
378 1-3). Thus, the effect of soil fertility on leaf quality and leaf damage varied among flush stages.
379 These intriguing patterns may be understood in terms of that the timing of herbivory could

influence induced responses. For example, during the elongation of the first flush, leaf damage was caused mainly by the larvae of Lepidoptera, which feed on leaves before the leaves have finished unfolding. While, during the elongation of the second flush, leaf damage was caused by the adults of Coleoptera, which feed on leaves after the leaves have finished unfolding. The leaf properties, such as CT and TP concentration and LMA, might be affected by at what stage of leaf expansion did herbivory occur. Our experimental set-up in this study was such that the sampling of all leaves was conducted at one time at the end of the growing season. Sequential sampling in accordance with leaf expansion may allow us to examine whether the higher induced defence observed in some treatments has the potential to protect the plants better against subsequent insect damage.

We previously demonstrated that in high-fertility soil the probabilities of producing subsequent shoots were higher (Mizumachi et al. 2006), and the number of shoots and total shoot length produced in one growing season were greater (Mizumachi et al. 2004). It means soil fertility affected not only the concentration of CT and TP in leaves, but also the growth patterns of *Q. serrata* seedlings (Mizumachi et al. 2004, 2006). In low-fertility soil, the contribution of the first shoots to the growth within one growing season is relatively greater than

396 in high-fertility soil. In the present study, seedlings in low-fertility soil were better defended
397 against herbivore damage than those in high-fertility soil (Figs. 1–3). Taken together, these
398 results suggest that *Q. serrata* seedlings adjust the balance between induced response and
399 growth according to resource availability: seedlings in low-fertility soil have higher level of
400 induced response while showing relatively low potential for production of new photosynthetic
401 organs compared to those growing on more fertile soil.

402 In summary, this and our previous studies (Mizumachi et al. 2004, 2006) showed that *Q.*
403 *serrata* seedlings have the ability to produce later shoots and the ability to strengthen defensive
404 properties of leaves against herbivore damage. Furthermore, *Q. serrata* seedlings appear to
405 adjust the balance between these abilities according to resource availability. Given that
406 invertebrate herbivores prefer seedlings over older woody plants (Boege and Marquis 2005) and
407 that *Q. serrata* is host to 346 lepidopterans species (Teramoto 1996), the potential of *Q.*
408 *serrata* seedlings to adjust their physiological and morphological properties depending on
409 herbivory and soil fertility can be advantageous in forest floor where seedlings constantly are
410 exposed to herbivory.

411

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421

422 **References**

423

424 Alaoui-Sossé B, Ricaud S, Barnola P, Dizengremel P (1996) Rhythmic growth and carbon
425 allocation in *Quercus robur*. Sucrose metabolizing enzymes in leaves. Physiologia Plant
426 96:667-673

427 Baraza E, Gómez JM, Hódar JA, Zamora R (2004) Herbivory has a greater impact in shade than

in sun: response of *Quercus pyrenaica* seedlings to multifactorial environmental variation. Can J Bot 82:357-364

Boege K (2004) Induced responses in three tropical dry forest plant species – direct and indirect effects on herbivory. Oikos 107:541-548

Boege K, Marquis RJ (2005) Facing herbivory as you grow up: the ontogeny of resistance in plants. Trends Ecol Evol 20:441-448

Borchert R (1975) Endogenous shoot growth rhythms and indeterminate shoot growth in oak. Physiol Plant 35:152-157

Chaar H, Colin F, Collet C (1997a) Effects of environmental factors on the shoot development of *Quercus petraea* seedlings -A methodological approach. For Ecol Manage 97:119-131

Chaar H, Colin F, Leborgne G (1997b) Artificial defoliation, decapitation of the terminal bud, and removal of the apical tip of the shoot in sessile oak seedlings and consequences on subsequent growth. Can J For Res 27:1614-1621

Choong MF (1996) What makes a leaf tough and how this affects the pattern of *Castanopsis fissa* leaf consumption by caterpillars. Funct Ecol 10:668-674

Coley PD (1983) Herbivory and defensive characteristics of tree species in a lowland tropical

444 forest. Ecol Monogr 53:209-233

445 Cornelissen T, Stiling P (2006) Does low nutritional quality act as a plant defence? An

446 experimental test of the slow-growth, high-mortality hypothesis. Ecol Entomol 31:32-40

447 Dickson RE, Tomlinson PT, Isebrands JG (2000) Allocation of current photosynthate and

448 changes in tissue dry weight within northern red oak seedlings: individual leaf and flush

449 carbon contribution during episodic growth. Can J For Res 30:1296-1307

450 Feeny P (1970) Seasonal changes in oak leaf tannins and nutrients as a cause of spring feeding

451 by winter moth caterpillars. Ecology 51: 565-581

452 Forkner RE, Hunter MD (2000) What goes up must come down? Nutrient addition and

453 predation pressure on oak herbivores. Ecology 81:1588-1600

454 Forkner RE, Marquis RJ (2004) Uneven-aged and even-aged logging alter foliar phenolics of

455 oak trees remaining in forested habitat matrix. For Ecol Manage 199:21-37

456 Forkner RE, Marquis RJ, Lill JT (2004) Feeny revisited: condensed tannins as anti-herbivore

457 defences in leaf-chewing herbivore communities of *Quercus*. Ecol Entomol 29:174-187

458 Furuno T, Saito H (1981) Seasonal variations of litter fall and primary consumption by

459 herbivorous insects in *Quercus serrata* in Kyoto (in Japanese). Bull Kyoto University Forest

460 53: 52-64

461 Hanley ME, Lamont BB, Fairbanks MM, Rafferty CM (2007) Plant structural traits and their

462 role in anti-herbivore defence. Perspectives in Plant Ecol, Evol and Systematics 8:157–178

463 Haukioja E (1990) Induction of defenses in trees. Annu Rev Entomol 36:25-42

464 Hikosaka K, Takashima T, Kabeya D, Hirose T, Kamata N (2005) Biomass allocation and leaf

465 chemical defence in defoliated seedlings of *Quercus serrata* with respect to carbon-nitrogen

466 balance. Ann Bot 95:1025-1032

467 Hunter MD (1987) Opposing effects of spring defoliation on late season oak caterpillars. Ecol

468 Entomol 12:373-382

469 Hunter MD, Schultz JC (1995) Fertilization mitigates chemical induction and herbivore

470 responses within damaged oak trees. Ecology 76:1226-1232

471 Karban R, Baldwin IT (1997) Induced responses to herbivory. The University of Chicago Press,

472 Chicago, IL

473 Karban R, Myers JH (1989) Induced plant responses to herbivory. Annu Rev Ecol Syst

474 20:331-348

475 Koike T, Tobita H, Shibta T, Matsuki S, Konno K, Kitao M, Yamashita N, Maruyama Y (2006)

476 Defense characteristics of seral deciduous broad-leaved tree seedlings grown under differing
477 levels of CO_2 and nitrogen. Popul Ecol 48:23-29

478 Koricheva J, Larsson S, Haukioja E, Keinänen M (1998) Regulation of woody plant secondary
479 metabolism by resource availability: hypothesis testing by means of meta-analysis. Oikos
480 83:212-226

481 Kudo G (1996) Herbivory pattern and induced responses to simulated herbivory in *Quercus*
482 *mongolica* var. *grosseserrata*. Ecol Res 11:283-289

483 Kytö M, Niemelä P, Larsson S (1996) Insects on trees: population and individual response to
484 fertilization. Oikos 75:148-159

485 Lill JT, Marquis RJ (2001) The effects of leaf quality on herbivore performance and attack from
486 natural enemies. Oecologia 126:418-428

487 Lower SS, Kirshenbaum S, Orians CM (2003) Preference and performance of a willow-feeding
488 leaf beetle: soil nutrient and flooding effects on host quality. Oecologia 136: 402-411

489 Matsuki S, Sano Y, Koike T (2004) Chemical and physical defence in early and late leaves in
490 three heterophyllous birch species native to northern Japan. Ann Bot 93:141-147

491 Mattson WJ (1980) Herbivory in relation to plant nitrogen content. Annu Rev Ecol Syst

11:119-161

Mauffette Y, Oechel WC (1989) Seasonal variation in leaf chemistry of the coast live oak *Quercus agrifolia* and implications for the California oak moth *Phryganidia californica*. Oecologia 79:439-445

Migita C, Chiba Y, Tange T (2007) Seasonal and spatial variations in leaf nitrogen content and resorption in a *Quercus serrata* canopy. Tree Physiol 27:63-70

Mizumachi E, Mori A, Osawa N, Akiyama R, Tokuchi N (2006) Shoot development and extension of *Quercus serrata* saplings in response to insect damage and nutrient conditions. Ann Bot 98:219-226

Mizumachi E, Osawa N, Akiyama R, Tokuchi N (2004) The effects of herbivory and soil fertility on the growth patterns of *Quercus serrata* and *Q. crispula* saplings at the shoot and individual levels. Popul Ecol 46:203-211

Murakami M, Yoshida K, Hara H, Toda MJ (2005) Spatio-temporal variation in Lepidopteran larval assemblages associated with oak, *Quercus crispula*: the importance of leaf quality. Ecol Entomol 30: 521-531

Mutikainen P, Walls M, Ovaska J, Keinänen M, Julkunen-Tiitto R, Vapaavuori E (2000)

508 Herbivore resistance in *Betula pendula*: effect of fertilization, defoliation, and plant
509 genotype. Ecology 81:49-65

510 Nabeshima E, Murakami M, Hiura T (2001) Effects of herbivory and light conditions on
511 induced defense in *Quercus crispula*. J Plant Res 114:403-409

512 Nakamura M, Hina T, Nabeshima E, Hiura T (2008) Do spatial variation in leaf traits and
513 herbivory within a canopy respond to selective cutting and fertilization? Can J For Res
514 38:1603-1610

515 Nomura M, Itioka T (2002) Effects of synthesized tannin on the growth and survival of a
516 generalist herbivorous insect, the common cutworm, *Spodoptera litura* Fabricius
517 (Lepidoptera: Noctuidae). Appl Entomol Zool 37:285-289

518 Nykänen H, Koricheva J (2004) Damage-induced changes in woody plants and their effects on
519 insect herbivore performance: a meta-analysis. Oikos 104:247-268

520 Ohsawa T, Saito Y, Sawada H, Ide Y (2008) Impact of altitude and topography on the genetic
521 diversity of Quercus serrata populations in the Chichibu Mountains, central Japan. Flora
522 203:187–196

523 Ozawa H, Itoh K, Hori Y (2000) Shoot structure and dynamics of saplings and canopies of three

524 deciduous broad-leaved trees of a coppice forest in central Japan. Trees 14:206–214

525 Poorter H, Niinemets U, Poorter L, Wright IJ, Villar R (2009) Causes and consequences of

526 variation in leaf mass per area (LMA): a meta-analysis. New Phytologist 182:565–588.

527 Porter LJ, Hrstich LN, Chan BG (1986) The conversion of procyanidins and prodelphinidins to

528 cyanidin and delphinidin. Phytochemistry 25:223-230

529 Reich PB, Uhl C, Walters MB, Elsworth DS (1991) Leaf lifespan as a determinant of leaf

530 structure and function among 23 amazonian tree species. Oecologia 86:16-24

531 Rossiter MC, Schultz JC, Baldwin IT (1988) Relationships among defoliation, red oak

532 phenolics, and gypsy moth growth and reproduction. Ecology 69:267-277

533 Ruohomäki K, Chapin III FS, Haukioja E, Neuvonen S, Suomela J (1996) Delayed inducible

534 resistance in mountain birch in response to fertilization and shade. Ecology 77:2302-2311

535 Salminen J-P, Roslin T, Karonen M, Sinkkonen J, Pihlaja K, Pulkkinen P (2004) Seasonal

536 variation in the content of hydrolyzable tannins, flavonoid glycosides, and

537 proanthocyanidins in oak leaves. J Chem Ecol 30:1693-1711

538 SAS Institute (2005) JMP Statistical Discovery Software (ver. 6.0). SAS Institute Inc, Cary, NC

539 Southwood TRE (1961) The number of species of insect associated with various trees. J Anim

Ecol 30:1-8

Sprugel DG, Hinckley TM, Schaap W (1991) The theory and practice of branch autonomy. Annu Rev Ecol Syst 22:309-334

Stamp N (2003) Out of the quagmire of plant defense hypotheses. Q Rev Biol 78:23-55

Teramoto N (1993) Catalogue of host plants of lepidopterous insects in Japan (Fagaceae) (in Japanese). Bull Shiga Agric Exp Stn [Extra issue] 1:1-161

Teramoto N (1996) Studies on lepidopterous insect fauna on Fagaceous plants, as the food plants of the wild silk moth, *Antheraea yamamai* (in Japanese). Special Bull Shiga Agric Exp Stn 19:1-216

Wait DA, Jones CG, Coleman JS (1998) Effects of nitrogen fertilization on leaf chemistry and beetle feeding are mediated by leaf development. Oikos 82: 502-514

Waterman PG, Mole S (1994) Analysis of phenolic plant metabolites. Blackwell Science Publications, London

Watson MA (1986) Integrated physiological units in plants. Trends Ecol Evol 1:119-123

White J (1979) The plant as a metapopulation. Annu Rev Ecol Syst 10:109-145

Wold EN, Marquis RJ (1997) Induced defense in white oak: effects on herbivores and consequences for the plant. Ecology 78:1356-1369

557 Yamasaki M, Kikuzawa K (2003) Temporal and spatial variations in leaf herbivory within a
558 canopy of *Fagus crenata*. Oecologia 137:226-232

559

560

560 **Fig. 1** Condensed tannin (CT) concentration (means ± s.e.) in leaves of shoots produced
561 at each flush stage in *Quercus serrata* seedlings in treatments of herbivory and
562 soil fertility.
563
564 **Fig. 2** Total phenolics (TP) concentration (means ± s.e.) in leaves of shoots produced at
565 each flush stage in *Quercus serrata* seedlings in treatments of herbivory and soil
566 fertility.
567
568 **Fig. 3** Leaf mass per area (LMA; means ± s.e.) in leaves of shoots produced at each flush stage
569 in *Quercus serrata* seedlings in treatments of herbivory and soil fertility. Different letters
570 are significantly different by Tukey's HSD test ($P < 0.05$).
571
572 **Fig. 4** Nitrogen (N) concentration (means ± s.e.) in leaves of shoots produced at each
573 flush stage in *Quercus serrata* seedlings in treatments of herbivory and soil
574 fertility.
575

576 **Fig. 5** Leaf damage class (means ± s.e.) of shoots produced at each flush stage in

577 *Quercus serrata* seedlings in treatments of soil fertility.

Table 1 Leaf properties (means ± standard error) of shoots produced each flush period in *Quercus serrata* seedlings subjected to herbivore damage at various soil nutrient levels

Leaf property		Flush period				*P*
		First flush		Later flush		
CT	Damaged-Low	10.19 ± 0.82	(n = 13)	4.99 ± 1.05	(n = 8)	0.0010
	Damaged-High	7.31 ± 0.47	(n = 13)	4.19 ± 0.54	(n = 10)	0.0003
	Undamaged-Low	5.97 ± 0.36	(n = 14)	2.54 ± 0.47	(n = 8)	< 0.0001
	Undamaged-High	3.81 ± 0.48	(n = 13)	3.07 ± 0.36	(n = 23)	0.2279
LMA	Damaged-Low	6.19 ± 0.20	(n = 21)	6.83 ± 0.17	(n = 28)	0.0169
	Damaged-High	5.38 ± 0.17	(n = 29)	5.87 ± 0.22	(n = 18)	0.0871
	Undamaged-Low	4.91 ± 0.07	(n = 51)	4.94 ± 0.15	(n = 10)	0.8704
	Undamaged-High	5.15 ± 0.16	(n = 20)	5.47 ± 0.11	(n = 43)	0.1157

Differences were tested with Student's *t*-test.

CT, condensed tannin content in leaves; LMA, leaf mass per area.

Fig. 1

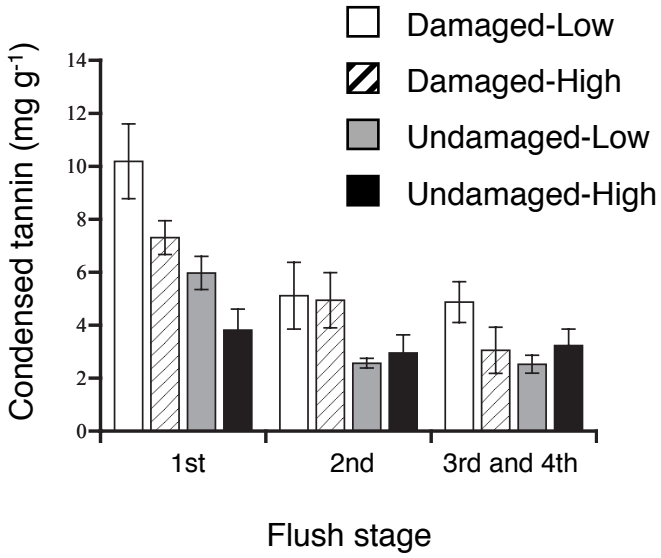

Fig. 2

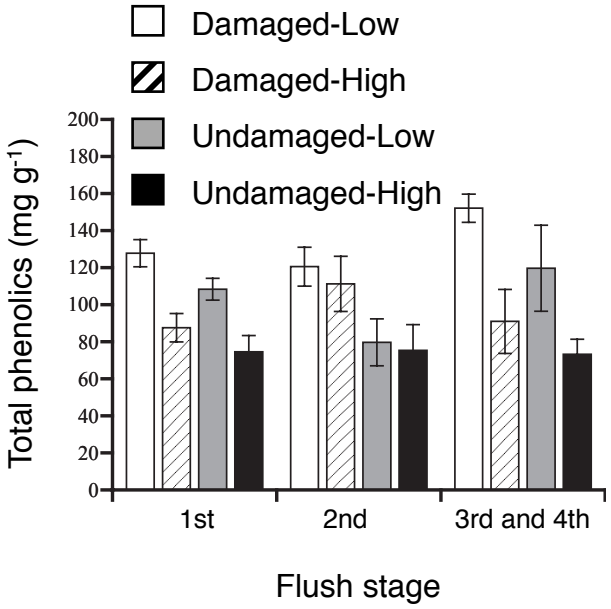

Fig. 3

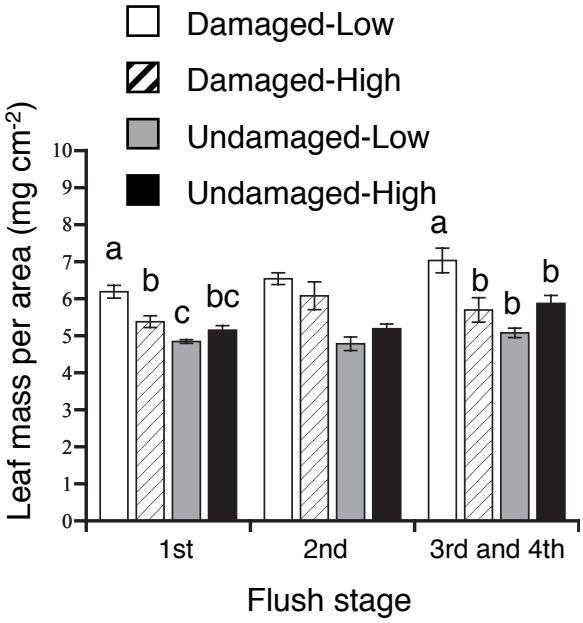

Fig. 4

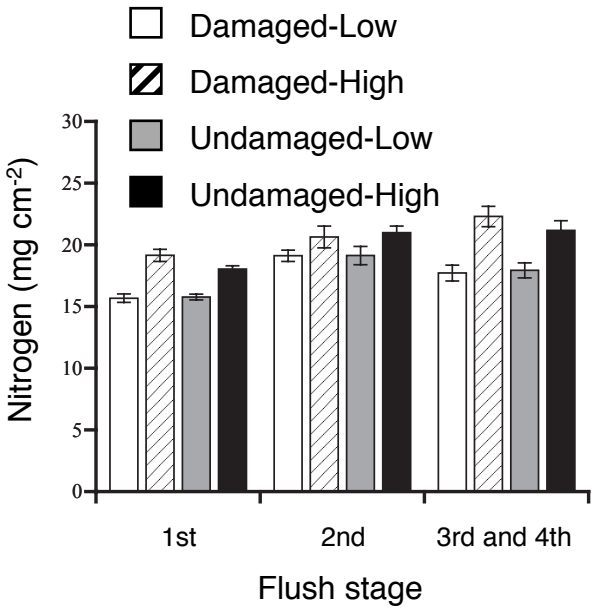

Fig. 5